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Methods Used to Control Pollination of Pines in the Sierra Nevada of California

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INTRODUCTION

Controlling pollination is the only way to produce hybrid plants of known parentage. This permits the breeding of new plant forms with characteristics of special value to man. In forest-tree breeding, controlled pollination has produced hybrid trees showing such desirable characteristics as vigor, good form, and resistance to insects, drought, or cold. Through these new forms, tree breeders may produce more wood per acre per year, reduce the time required to produce merchantable timber, or develop trees adapted to areas now producing no timber.

This circular describes techniques devised for controlling pollination in *Pinus* under conditions prevailing in the Sierra Nevada of California. Developed during a period of 20 years,[2] they have proved effective and practicable when used correctly; properly modified, these techniques may be applied to other cone-bearing trees in other regions.

[1] Maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California, Berkeley, Calif.

[2] The following present or former members of the staff of the Institute of Forest Genetics, Placerville, Calif.—formerly the Eddy Tree Breeding Station—participated in developing the techniques: Lloyd Austin, J. S. Barnes, C. R. Berriman, W. C. Cumming, Earl J. Hodgkins, Emery F. Kimbrough, F. I. Righter, and W. G. Wahlenberg. Photography by A. R. Liddicoet, also of Institute staff.

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To insure positive and selective pollination in the pines, effective techniques must provide for: (1) Isolating ovulate flowers, (2) collecting and preparing pollen for use, (3) pollinating, (4) protecting cones and seeds. Some of the techniques devised by the institute for these purposes have been described or illustrated previously (*1*, *2*, *21*).[3] For the sake of completeness, they are either referred to or described fully in this circular.

ISOLATING THE OVULATE FLOWERS

In *Pinus*, ovulate strobili develop during a period of 3 to 4 weeks from a tiny bud to a conelet ready for pollination. Since the steps in controlling pollination must be synchronized with developmental stages (fig. 1), it has been advantageous to arbitrarily recognize seven

FIGURE 1.—Stages in the development of the ovulate strobilus of *Pinus Jeffreyi*. At "flowers maximum," the optimum stage for pollination, the cone scales (not the spines) extend roughly at right angles to the axis of the conelet.

[3] Italic numbers in parentheses refer to Literature Cited, p. 17.

stages in this development: (1) Buds small, (2) buds large, (3) buds opening, (4) flowers partly open,[4] (5) flowers maximum, (6) flowers closed, (7) cones enlarging. Stages 1, 2, and 3 are determined by the size or condition of the bud. Stages 4, 5, 6, and 7 are determined by the position of the cone scale relative to the axis of the conelet. In stage 6, the cone scales are tightly closed so that pollination is no longer possible.

In the transect of the Sierra Nevada from Sacramento through El Dorado County, *Pinus ponderosa* occurs over an elevational range of approximately 6,800 feet (100–6,900 feet). On comparable aspects, trees at low elevations mature their flowers earlier than do those at high elevations. Thus, pollen from trees at low elevations may be in the air several weeks before trees at higher elevations mature their ovulate flowers. Between 2,200 feet and 4,000 feet, there is a span of approximately 35 days over which *P. ponderosa* sheds its pollen. Other species of pine behave similarly.

It is desirable to isolate the ovulate flowers when there is little or no pollen in the air. Accordingly, they are enclosed in bags as soon as possible after their buds have grown out of the fibrous covering of the branch terminal—usually at the "buds small" stage, though sometimes at the "buds large" stage. Bagging at those stages is also recommended by Dengler (*8*), who conducted breeding tests with *P. banksiana*, *P. montana*, and *P. Sylvestris*. Snow, Dorman, and Schopmeyer (*25*), however, consider it safe in northern Florida to bag *P. caribaea* up to and including the "buds opening" stage.

Ovulate flowers are usually isolated by enclosing them individually or in clusters in pollenproof bags (pollination bags), though Syrach Larsen (*27*) has enclosed large parts of trees or even entire trees (up to 7.2 m. in height) in tents. Pollination bags are made of various materials, depending on the genus involved, the conditions to which the bags are exposed, and cost of the materials. Various kinds of paper bags have been used on conifers by Austin (*4*, *5*), Dengler (*8*), Johnson and Bradley (*17*), Munns (*18*), Schreiner (*22*), Syrach Larsen (*27*), and others; and on various kinds of angiospermous trees by Henry (*13*), Johnson and Bradley (*17*), Schreiner (*22*), Stout, McKee, and Schreiner (*26*), Piatnitsky (*20*), and others. Austin (*5*), Syrach Larsen (*28*), and Traub and Romberg, (*29*), have used various kinds of plain and paraffined cloth bags. Johnson and Bradley (*17*), Smith and Romberg (*24*), Schreiner (*22*) have used bags made of cellophane or glassine. Smith and Romberg (*24*) recommend viscose sausage casings for use on pecans. Paper bags have the advantage of being light and inexpensive. Glassine, cellophane, and sausage-casing bags have those advantages, and are also transparent.

Cumming (*7*) found that bags of light cloth (plain or paraffined), paper (plain or waxed), cellophane, glassine, Vio Ray, and glass cloth are not suited to Sierra Nevada conditions, mainly because they lack requisite toughness. In moisture-tight bags, moreover, vapor from transpiration may condense on, and obstruct vision through, transparent substances. Furthermore, the weight of the water that accumulates in such bags may cause the limbs of some species to bend down and sometimes break off. Tests recently initiated by J. W.

[4] The term "flower" is used here in its broad meaning as a reproductive organ since the conifers are not classified as flowering plants.

Duffield [5] indicate that sausage casings, which are pervious to moisture, may be adapted to much of the institute's work.

The method used by Syrach Larsen (*27*), Schreiner (*22*), and Johnson and Bradley (*17*) of enclosing a transparent (glassine or cellophane) bag in a larger kraft bag for protection may be suitable for some of the work in the Eddy Arboretum at Placerville, but it is not deemed to be sufficiently reliable for use on large trees in the forests.

The Pollination Bag

The institute's standard bag (fig. 2), which is 14.5 inches by 8 inches, is constructed of a piece of finely woven 10- or 12-ounce Army duck of the following dimensions: Overlap section, width—9 inches, length—7.25 inches; overlapped section, width—8 inches, length—7.25 inches. A durable, waterproof fabric cement holds its seams. Staples at the corners of the bag reinforce the cement and increase the rigidity of the bag. A 3- by 4-inch window, made of cellulose acetate (0.01 inch thick) and cemented in place, permits inspection of the flowers without removing the bag.[6]

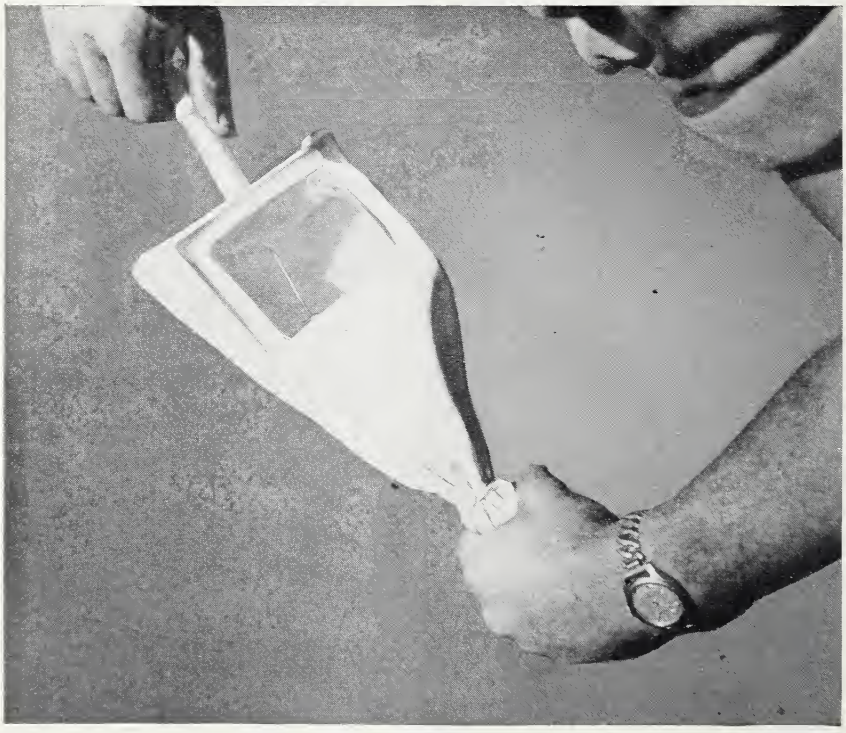

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FIGURE 2.—Pollination bag and hypodermic syringe used at the Institute of Forest Genetics.

[5] Geneticist, Institute of Forest Genetics, Placerville, Calif.

[6] Complete directions for making the bag are given by A. R. Liddicoet in Pinus: Pollination Bag Construction, California Forest and Range Experiment Station Forest Research Note 47. 4 pp., illus. [Processed.]

The bags are too heavy for use on some trees—at least while the trees are young and growing rapidly—unless provision is made to support the branch in its normal position. For example, the long shoots of young (5 to 20 years of age) rapidly growing trees of *Pinus Jeffreyi*, *P. lambertiana*, *P. murrayana*, *P. monticola*, and *P. ponderosa* may require support, but the shorter shoots of large, mature, more slowly growing trees of these species easily support the weight of the bags. The extent to which this difficulty would be encountered on large trees of the various species of pine is unknown. Such trees of most species have not been available for trial. The difficulty might be encountered even on old trees of species such as *P. glabra* and *P. halepensis*, which have relatively slender shoots. It can be overcome by installing a grommet in one of the upper corners of the bag or using one of the staples as a grommet so that the bag can be held up by a piece of string or wire attached to some part of the tree above the bag.

This bag is not ordinarily injured by rain, hail, thrashing by wind, and the rough handling which speedy work in large trees necessitates. It endures without injury a temperature of 82° F. for 15 hours, which is sufficient to kill pine pollen. Its initial cost is comparatively high, but because it can be used many times, in the long run it probably compares favorably in cost with a bag that can be used only once.

Before being used, the bag is cleaned with a vacuum cleaner, and wrinkles formed at the open end during previous use are removed by ironing. A 3- by 6-inch strip of cotton about one-half inch thick, which will be wrapped around the branch at the point of attachment of the bag, is then placed in the bag, and the bag is sealed with gummed paper. The bag is then subjected to heat (80°–82° F.) for 15 hours to kill any viable pollen that may be in it (*14*). Immediately prior to use, the bag is inspected for defects such as openings in the seams and around the window and for cracks or holes in the window. The window material sometimes deteriorates in from 3 to 5 years and must be replaced.

Bagging the Flowers

Equipment used in bagging—some of it is used in later operations also—consists of pollination bags, 25-inch lengths of 16-ply cotton cord for tying the bags to the branches, pencils, a notebook with record sheets (fig. 3), a knapsack in which to carry the foregoing items, a 100-foot length of half-inch rope, and an 8-foot length of stiff No. 6 wire with a 4-inch hook bent in one end.

Breeding work is done on trees of all sizes, from a few feet to 150 feet or more in height. Large trees (4 feet d. b. h. and over), however, offer several advantages. Because of its greater reproductive capacity, a 130-foot ponderosa pine may accommodate from 10 to 20 times as many pollination bags as a 60-foot ponderosa pine. Moreover, it is easier and safer to work in the tops of such trees because of the character of the upper part of their crowns, where, in *Pinus*, most of the ovulate strobili are borne (*23*).

The crowns of these large trees, besides being comparatively widespread, are usually rounded or flat and are often composed of a great multitude of comparatively short shoots. Hence it is frequently pos-

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POLLINATION RECORD

19____

Experiment number _____ Seed parent _____

Flowers bagged _____ Maturity _____ Disbagged _____

Cones bagged _____ Collected _____

Tag number	Number of flowers	Pollination data				Crop	
		Pollen parent, with year of pollen collection	Pollinations			Cones bagged	Cones collected
			Date	Maturity	By		

FIGURE 3.—Form used by the Institute of Forest Genetics to record pollination and crop data.

sible to put on from 10 to 20 pollination bags, or to pollinate them, without changing one's position in the tree. The shortness of the shoots—the result of a slow rate of elongation—is itself an advantage, for the terminals are not as easily broken as are the longer terminals of young, rapidly growing trees. Limbs are stout and strong, even at the tops of large trees, and owing to the slowness of growth, the limbs, which are more or less staggered, grow in closely spaced whorls. As these limbs break up into numerous branches bearing many stout terminals, there is very little danger that a man of normal reflexes would fall out of such a tree. Thus, the nature of the crown affects the speed and safety with which work can be done. These advantages are somewhat offset, however, by the difficulty of climbing large trees.

The first step in bagging the flowers is to climb the tree. Several alternative methods are possible. The usual method, if branches cannot be reached from the ground, is to throw the rope over a branch and climb the rope (fig. 4). The knapsack may be carried up or pulled up later with the rope.

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FIGURE 4.—Footlock method of climbing a rope used in tree-breeding work. *A*, Footlock, supporting climber, has been made and the climber is ready to pull himself higher. *B*, The pull-up is completed and the right foot is winding the rope around the instep of the left foot to establish the supporting "lock."

As each tree must be climbed eight or more times in the course of a single breeding experiment, the use of climbing irons, which causes considerable bark injury, is not feasible.

Portable ladders, though somewhat cumbersome, can also be used. Even ladders built on trees of exceptional importance may be practicable. In Sweden scaffolds have been erected around large trees of exceptional interest such as elite trees and polyploids (*3*), enabling workers to reach all parts of the crown without standing on limbs. Dorman, Schopmeyer, and Snow (*10*) prevent breakage of, and provide safe, efficient working conditions on, young trees of *Pinus caribaea* and *P. palustris* by bracing the trees with three 10-foot to 16-foot pieces of 2- by 2-inch pine. Using a helicopter to reach the tops of

large trees may be feasible at some future date. Such an aid would also be invaluable for reconnaissance work.[7]

The number of flowers borne by individual branches varies on most trees so that there usually is a choice respecting the number of flowers that can be enclosed in any bag. Usually, bagging is done so as to include as many flowers per bag as possible. If bagging of a specified number of flowers is required, this course may make it possible to use fewer bags and thereby reduce the amount of field work to be done; or, if a specified number of bags are to be used, to obtain more seed than would otherwise be obtained.

Experimental requirements, however, may sometimes justify the sacrifice of those advantages. For example, certain kinds of tests may require that each bag enclose the same number of flowers. Then, too, if the test includes self-pollinations, the bags for such pollinations should be allocated to those buds which show the lesser development. Thus, if there is a choice of bagging five or six flowers at the "buds large" stage or one or two flowers at the "buds small" stage, the latter course should be chosen because the more advanced flowers may pass the receptive stage before pollen from the same tree can be obtained and prepared for use. On the other hand, buds that are at an early buds-small stage when others on the same shoot are at a buds-large stage frequently do not mature. Such buds should not be depended upon.

Branches at the top of a tree can often be bagged without the aid of the wire hook, but long branches must be pulled in with the hook and held in place by bending the wire around another branch. Needles are then removed from the place on the branch where the bag is to be attached.

In bagging species having long needles, it is advisable to remove most of the needles beyond the point of attachment. This facilitates the work, insures that flowers will not be buried in a mass of foliage, and reduces transpiration which might otherwise result in excessive condensation of vapor on the window. Usually, all the needles on two sides of the tip and all but four or five fascicles on the other two sides are pulled off. On some *Pinus jeffreyi* trees, pulling the needles off may result in stripping of the bark unless the needles are removed a few at a time and with considerable care. Johnson and Bradley (*17*) trim the needles with scissors, a method which might be advantageous on such trees. On stiff-needled species trimming of needles results in a protective ring that keeps the tender conelet from rubbing against the bag. However, this method is not used at the institute.

All pollen catkins that would be enclosed in the bag should be removed unless self-pollination is desired.

When the needles and catkins have been removed, the bag is opened and the cotton is placed in position. Workers hold the bag with their teeth while wrapping cotton around the branch. The branch is inserted into the bag so that needles lie next to the seams. Then the bag is closed tightly with a piece of string tied in a clove hitch. The leader with buds should be in the center of the bag, below or near the bottom of the window, and the bag should be well rounded out, as

[7] Means of rendering the work less strenuous as well as less dangerous are being investigated.

though inflated, with the closed end pushed in to keep the bag expanded.

The number of flower buds enclosed in each bag is recorded so that the worker can ascertain whether or not a reasonable surplus of buds over the desired number has been bagged.

If there is no surplus, a few more bags should be put on to compensate for failure of buds to mature, bagged branches breaking off, etc. Moreover, such records enable a count of flowers that develop into cones. It is also advisable to designate the flowers that are to be used as checks by tagging them at this time. These checks provide for (1) a comparison of the percents of bagged and unbagged flowers which reach maturity, (2) a comparison of the efficacy of wind and hand pollination, and (3) seed of known origin to be used in nursery tests.

POLLEN HANDLING

In general, there are two methods of collecting pollen: (1) Bagging catkins before they ripen, (2) detaching catkin-bearing branches or detaching the catkins alone.

The first method, which is not used at the institute, requires bags of smooth materials (waxed paper, glassine, etc.) because pollen does not adhere to such materials if they are dry, as it does to cloth materials. But since smooth materials are impervious to moisture, vapor from transpiration may condense on the inside of the bag and wet the pollen when it sheds. Usually, only a few catkins can be enclosed in a bag so that many bags and much work are necessary to obtain enough pollen. If cloth bags are used, more of them are required because extraction of pollen from them is incomplete. Finally, windborne pollen may get into the bags when they are removed from the branches.

The second method, of which there are various modifications, is used in most tree-breeding work. Barnes picked catkins a day or two before they were ripe. This method was used by Traub and Romberg (*29*) with pecans. Syrach Larsen (*27*) even shot down catkin-bearing twigs of a large larch tree. More recently, Schreiner (*22*) recommended cutting catkin-bearing branches and keeping them in water in the laboratory until the catkins mature, a method used by several other investigators. This method is cumbersome and not adapted to the collection of large quantities of pollen.

Collecting Catkins

A modification of the second method is used by the Institute of Forest Genetics. It is essentially that used by Barnes (*6*) and by Traub and Romberg (*29*). As soon as the tree begins to shed its pollen, unopened catkins are picked, put in paper bags, and taken to the laboratory. If a tree that has not commenced to shed is far from the laboratory, its catkins are examined with a field microscope to ascertain their stage of development. If aceto-carmine smears show that pollen grains have completed the second division in the development of the gametophyte (remnants of two prothallial cells will be visible), the catkins will ripen after picking whereas catkins collected prior to that stage may not ripen. Such examination may enable the investigator to avoid a long return trip to the tree for pollen.

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Extracting Pollen

Pollen is often obtained from untreated catkins by catching it in paper trays or other suitable receptacles as it sheds from the catkins in the laboratory. Traub and Romberg (*29*) carefully washed unopened catkins in running water, wiped them off with a sterile towel, and then allowed them to dry and shed their pollen in a desiccator after which the pollen was sifted through a fine mesh sieve. If rigid control of pollination in *Pinus* is desired, however, a higher refinement in pollen extraction is necessary. The following technique, which yields a volume of pollen amounting roughly to 10 percent of the volume of the catkins, has proved to be dependable and practicable in breeding pines at the institute.

THE POLLEN EXTRACTOR

The apparatus used for extracting pollen (fig. 5) consists of a pollenproof bag fitting tightly over a funnel that empties through a rubber tube into a glass receptacle.

The funnel may be any size—the institute's are 12 inches in diameter or larger. A 4-inch circle of 60-mesh screen is soldered within the funnel just above its spout to catch coarse materials, such as catkin particles, dried pitch, etc., which otherwise would be mixed with the pollen. A sieve of the same diameter as the funnel is held about 2 inches above the funnel by metal legs that are soldered to both the funnel and the sieve. This sieve consists of a hoop of light metal, 2 inches high, with a circle of ordinary window screen soldered to the bottom of the hoop.

The bag, similar in material and construction to the pollination bags, but having two windows to permit observation of the interior of the extractor, is 30 inches long and of a diameter to fit snugly over the top of the funnel.

The receptacle may be a vial, flask, or glass bottle equipped with a rubber cap in which a cross (+) is cut to permit entry of the rubber tube into the receptacle. When the tube is forced through the cut in the cap, the cap makes a tight fit around the tube. When the tube is withdrawn, the cap closes automatically. The grip of the cap on the tube is strong enough to sustain the weight of the receptacle and the pollen.

All this equipment is sterilized prior to use, and the bag is sealed until it is needed.

EXTRACTION PROCEDURE

Catkins are prepared for extraction by pouring them into the sieve, which will accommodate 1,200 cc. or more. A 12-inch circle of window screen is placed over the catkins to hold them in the sieve while they are being washed thoroughly. They are first washed in an empty tank by means of a strong stream of lukewarm water, to remove pollen which may be on the unopened catkins. The funnel is then transferred to an adjacent tank and completely submerged for 1 minute in lukewarm water that flows gently from the bottom of the tank up over the rim into a discharge trough. This washing is to remove any pollen that may have settled on the catkins or in the funnel after the stream of water is shut off. As pine pollen rises quickly to the surface of still water, a minute of such washing is sufficient.

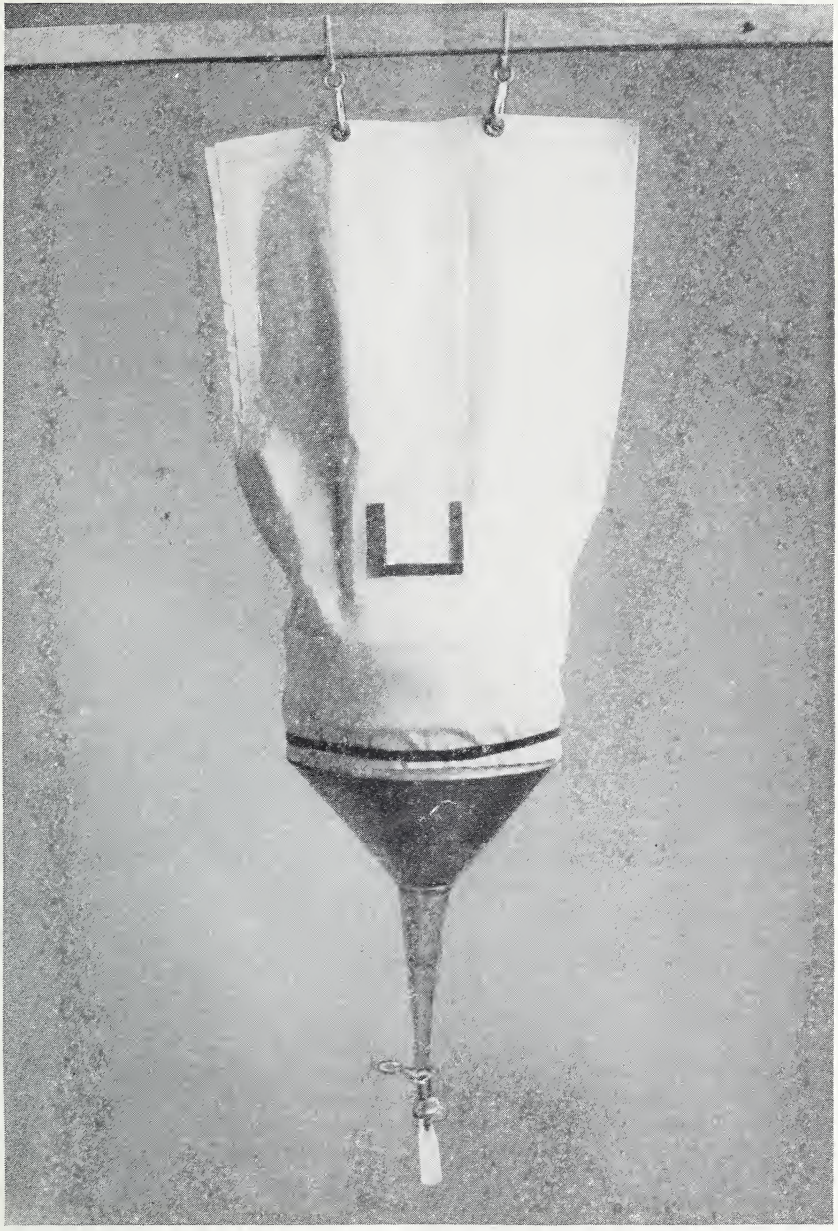

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FIGURE 5.—Pollen extractor in use.

While the catkins are still submerged, the lower part of the bag is placed in the water, and the seal is removed under water. The open end of the bag, while still under water, is stretched over the top of the submerged funnel and bound tightly with a strong rubber band

cut from an inner tube. The extractor is then removed from the tank and, after the water has drained out of it, a sterile cotton plug is inserted into the end of the rubber tube. The extractor is hung in a small, well-heated room in which the air is moved by an electric fan. After all the water has drained out, a metal clamp is attached to the tube for controlling the flow of pollen from the extractor.

From 1 to 3 days after the extractor is loaded, pollen is available. Before drawing off the pollen, the extractor is shaken vigorously. The cotton plug is removed from the tube, the cap on the flask and the end of the tube are wiped with 70-percent alcohol, and the tube is pushed through the cap. When the clamp is released, pollen flows into the flask. When the pollen has been drained from the extractor, the flask is pulled off the tube, and, after the cap has again been wiped with alcohol to remove or kill any foreign pollen, a sterile cotton plug is forced through the cap to seal the flask. The cap is then removed.

Occasional grains of foreign pollen may remain on the catkins or in the extractor, but no effective adulterations have been traced to this cause. Probably, the thorough wetting, followed by several hours in the warm, moist atmosphere of the extractor, causes these stray pollen grains to germinate. Then, as the equipment dries, they die.

Testing Pollen

Freshly collected pollen that has been properly handled is usually highly viable, but as a single method of handling may not yield uniform results when applied to different species, a collection of pollen may undergo rapid deterioration in viability from causes other than lapse of time. Therefore, in order to avoid both loss of time and uncertainty about results of breeding tests, each lot of pollen must be tested just prior to each use that is made of it. The method described by Righter (*21*) has proved convenient and effective.

Briefly, the method consists of three operations. A germination chamber is prepared first. To do this, melted paraffin is poured to cover the bottom of a Petri dish 2 or 3 mm. deep. Holes are punched out of the paraffin after it solidifies, permitting the germination cultures (hanging drops) to rest against clear glass. A few drops of distilled water are placed in the top of the Petri dish to maintain humidity in the chamber, and the inside periphery of the top section is coated with vaseline to provide a seal for the chamber.

The cultures are prepared next, using sterile hypodermic syringes equipped with rubber bulbs and 15-gage needles. A portion of each lot of pollen is withdrawn from its container into a syringe, and distilled water is next drawn into the syringe and mixed with the pollen by shaking. To place the culture in the Petri dish, a drop of the pollen-water mixture is squeezed out into a hole. Care must be taken in this operation to take up enough distilled water so that the mixture will not be too dense as well as to agitate the mixture before each drop is squeezed out.

After the holes are filled, the bottom of the dish is inverted and fitted into the top so as to effect an airtight seal. The dish is then placed in an electric oven, adjusted to maintain the temperature at about 26° C.

Although this method is convenient, it could be improved if Petri dishes having the holes precast could be obtained, thus eliminating much painstaking work in preparing the germination chamber.

Storing Pollen

With respect to viability, pine pollen is relatively durable and, even when kept in flasks in the laboratory, may retain a high degree of viability for months (*12*, *15*, *19*, *21*). Under such conditions, however, retention of viability is not certain and should never be assumed. If, as frequently happens, it is planned to use a lot of pollen from a week to a year after it ripens, it should be stored under conditions known to preserve its viability. Duffield and Snow (*12*), working with *Pinus resinosa* and *P. strobus* found that pollen stored one year in relative humidity of 50 percent at 0°–4° C. gave more than 80 percent germination. Johnson (*16*) found that after 12 months the best germination for pine pollen was with 50 to 75 percent relative humidity at 2° C. Dengler and Scamoni (*9*) report that an insecticide called effusan (formula not given) stimulated vigorous germination in pollen which otherwise exhibited little or no viability.

Viable pollen is not necessarily fertile. In most species of pine, approximately a year elapses between germination of the pollen grain in the micropyle and fertilization of the egg cell. Stored but viable pollen may not possess the necessary vigor to grow normally for a year in the nucellar tissue and then fertilize the egg when the latter is ready.

POLLINATING THE FLOWERS

Pollination in *Pinus* has been described by Doyle and O'Leary (*11*). Their discussion suggests that in *Pinus*, pollinating during the night might be more effective than daytime pollinating. But pollinating at night is impracticable on large trees and may be so on small trees. In any event, experimental results at various breeding stations have shown that daytime pollinating is effective.

The discussion of Doyle and O'Leary also suggests that in *Pinus* the position of the ovulate strobilus facilitates effective pollination. Normally the strobilus stands upright, or nearly so. In virtue of its wings, the pollen grain rises through the droplet in the micropylar canal and contacts the nucellus with the prothallial-cell side of the grain away from, and the pollen-tube side toward, the nucellus. Upon germination, the pollen tube comes directly in contact with the nucellus and can penetrate that tissue immediately. However, if the strobilus-bearing limb is bent very much, the resultant unnatural orientation may inhibit the normal progress of the pollen tube and prevent fertilization.

Pollination probably is effective from the fourth stage to the sixth stage of strobilus development, but doubtless it is most effective during the fifth, or "flowers maximum," stage, when all the cone scales stand at right angles to the axis of the strobilus. At this stage the body of the strobilus can be seen between the scales. Depending upon weather conditions, the period delimited by the fourth and sixth stages varies from 3 to 7 days for both bagged and unbagged flowers.

Various methods of pollinating have been reported. Some investigators removed pollination bags in order to pollinate; but it pollen is in the air at the time of pollinating, this procedure is not advisable. Consequently, at Placerville and elsewhere, methods of applying pollen without removing bags have been devised. Syrach Larsen (*27*), Schreiner (*22*), and Johnson and Bradley (*17*) remove the outer of two bags, puncture the inner bag with a medicine dropper or similar instrument, and blow the pollen into the bag with the rubber bulb of the dropper or with a special blower, and then replace the outer bag. Syrach Larsen (*27*) seals the designated pollen in the pipette part of the dropper by plugging the large end with cotton, which prevents the pollen from getting into the blower. The "glass points" or pipettes are carried in glass tubes closed with cotton plugs. The large end of the "glass point" is inserted into the rubber tube that leads from the bulb or blower. The point is discarded when its supply of pollen is exhausted, usually after a single pollination. The hole made in the bag is then closed with gummed paper and the outer bag is replaced. Though differing in details, the methods used by Schreiner (*22*) and Johnson and Bradley (*17*) are essentially similar to Syrach Larsen's.

Traub and Romberg (*29*) and the Institute of Forest Genetics (*1*, *2*) adapted hypodermic syringes to the purpose. The methods, though differing in details, are essentially similar.

Pollination Equipment

When the flowers begin to emerge from the buds, they must be watched closely so that they can be pollinated some time in the period extending from the fourth to the sixth stages and preferably during the fifth or "maximum" stage. Materials and equipment are then prepared for use. They include labeled vials of pollen, labeled hypodermic syringes (10 cc. capacity) equipped with rubber bulbs and 16-gage needles whose points are protected with corks or other coverings, tags bearing pertinent data, record sheets in a loose-leaf notebook, plungers for clearing needles that become plugged, a wire, a rope, pencils, and a knapsack. In some species (*Pinus lambertiana*, *P. monticola*, *P. murrayana*, and others) the work must be done mostly near the tops of the trees on small branches that extend almost at right angles from the trunk. On such trees, safety belts facilitate the work and enable the worker to avoid getting pitch on hands, clothing, and equipment. In most large trees of *P. jeffreyi* and *P. ponderosa*, as well as of many other species, safety belts would be cumbersome and are unnecessary. Goggles or glasses are sometimes used to protect the eyes from pine needles.

Pollination Procedure

When a bag is found in which one or more of the flowers are ready for pollination, the worker takes the syringe containing the designated pollen from his pocket or other container, removes the covering from the needle tip, and forces the needle into the bag from a point that permits him to direct the needle at the flowers. (See fig. 2, p. 4.) By manipulating the needle and the flowers, the worker usually can direct

the pollen at the flowers from almost any angle. A squeeze or two on the bulb expels sufficient pollen for the purpose. After withdrawing the needle and replacing the syringe in its container, the worker pinches or rubs the bag between his fingers to close the hole made by the needle. He then shakes the bag vigorously to distribute the pollen thoroughly around the flowers.

Next he fastens a properly labeled pyralin tag to the branch a few inches below the bag but above the whorl of branches produced the preceding year. The tag is 8 inches long. Stapled to it is a strip of mattress ticking 8 inches long to serve as a flag. The tag indicates that all cones maturing on the stem above it are included in the test designated on the tag.

After recording the number and maturity of the flowers in the bag and the date, the worker examines the other bags, treating similarly those containing any mature flowers. In some bags, all flowers may be ready for pollinating, but in others only one or two of the flowers may be ready. However, the other flowers in these bags will be approaching the proper stage, and, ordinarily, when this tree is worked over a few days later, they will be ready for pollination. Unless there has been a storm since the first pollination, these flowers may be pollinated merely by shaking the bags vigorously, filling the air with the excess pollen that has settled on stem, needles, etc., from the first pollination. It is advisable to repollinate such bags after a storm, however, since the excess pollen is likely to be caked or otherwise unsuitable for pollination. On trees bearing many bags, from three to five trips may be required to complete the pollinations.

When large quantities of seed are required of one particular hybrid, all the bags on from one to several trees should be assigned to the cross. This simplifies and reduces record taking, which otherwise consumes a large part of the total time required for pollinating.

Pollination is done under varying weather conditions, from cloudy to sunny and from calm to windy, but never during rain.

Removal of Pollination Bags

Pollination bags are removed after the ovulate flowers reach stage 7 (conelets enlarged). On fast-growing trees, bags should be removed as soon as possible after the conelets begin to enlarge; otherwise the leader will curl up inside the bag. This may injure both the ovulate flowers and the bag. On slow-growing trees, the bags are removed at some convenient time (usually from 10 days to 3 weeks after the last pollination has been made on the tree).

PROTECTING CONES AND SEEDS

During the current pollinating season it is necessary to protect the hand-pollinated pine conelets of the previous season from insects which attack cones or seeds. This protection is provided by enclosing the cones but not the tags in cloth bags (potato bags, sandbags, currency bags, etc.) that are large enough to contain mature, opened cones of the species on which they are used. These bags also protect the cones against squirrels, and catch the seeds from any cones that open before they are collected. If squirrels are working on bagged cones,

it is advisable to double bag and fasten the bags with wire rather than with a string.

Cones should be bagged before the insects which attack them become active. The usual practice is to bag the cones in the spring before they have enlarged appreciably; a bag is slipped over the end of the branch so that they are enclosed. As the pollination work must be done at the same time, either overtime work or sacrifice of tests already under way may be necessary. Elimination of cone bagging by leaving the pollination bags on the branches until the cones are ripe would prevent such troublesome conflicts and reduce the amount of work required very substantially. In some tests the bags would also serve the purpose of tags.

Recent observations suggest that pollination bags of the materials and construction described on page 4 can be left on the trees—without injury to bags or cones—until the cones are ready to be collected. The standard pollination bag is large enough to serve as a cone bag on species having small cones (*P. banksiana*, *P. echinata*, *P. murrayana*, and many others), but a bag of double its width would be required to accommodate several mature cones 5 inches long. Such a bag would accommodate straight shoot growth for 2 years on large old trees, but it would be too short to permit 2 years of straight shoot growth on young, vigorously growing trees of many species. In fact, it is too short for one season's growth on young trees of many species. However, crooked shoot growth is not particularly objectionable in itself, but only if it results in injury to the shoot, the ovulate flowers, or the bag. Conclusions regarding the optimum length of bag for particular species must await the results of further investigations.

TIME REQUIREMENTS

Travel required and other circumstances vary the time needed to carry out the operations described in this publication. Skilled men, working on large *Pinus ponderosa* and *P. jeffreyi* trees with the institute methods, carry out a 1,000-bag job in 30 to 45 man-days. Assuming an 8-hour day and 2 hours' travel per day for field work, the approximate time required for the various operations in such a job is:

Operation	*Man-days*
Prepare bags (clean, iron, seal, and sterilize)	4.00
Make tags	1.25
Label tags	.50
Reconnaissance	2.00
Bagging ovulate flowers (at 19 bags per hour)	9.00
Collecting and extracting pollen	1.00
Testing pollen	.10
Pollinating (at 10 bags per hour)	16.00
Removing bags	2.50
Bagging cones	4.00
Collecting cones	3.00
Cone and seed handling	2.00
Records (office)	.50
Total	45.85

Where all factors are favorable to production this estimate may be lowered by several days. Bagging flowers and pollinating, which together take over 50 percent of the total time, may vary considerably

with the individual agility of workers. If the crop of ovulate flowers is small, field operations require more time. Time required to bag cones is affected by the proportion of successful pollinations. These and other factors will cause variations from the estimate given above.

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